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RESEARCH ARTICLE

Chemometric-Assisted UV Method Development and Validation of Lamivudine and Zidovudine in Bulk and Tablet Formulation

P.Rajavel[1]*, S.Lavanya[2] and V.Manojkumar[3]

[1]Professor, Department of Pharmaceutical Analysis, Swamy Vivekanandha College of Pharmacy, Tiruchengode, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai), Tamil Nadu, India.
[2]M.Pharm Student, Department of Pharmaceutical Analysis, Swamy Vivekanandha College of Pharmacy, Tiruchengode, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai), Tamil Nadu, India.
[3]Assistant Professor, Department of Pharmaceutical Analysis, Swamy Vivekanandha College of Pharmacy, Tiruchengode, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai), Tamil Nadu, India.

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***Address for Correspondence**
P.Rajavel
Professor,
Department of Pharmaceutical Analysis,
Swamy Vivekanandha College of Pharmacy, Tiruchengode,
(Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai),
Tamil Nadu, India.
E.Mail: velraj512@gmail.com

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ABSTRACT

The study developed a chemometric-assisted UV method for estimating Lamivudine and Zidovudine. Standard drugs were dissolved in water, diluted, and scanned in the UV region. The method's precision was determined through intraday and inter-day analysis, with the average percentage recovery values being 98.6±0.2003 and 99.2±0.2042, respectively. The technique was useful to determine Lamivudine and Zidovudine in a tablet formulation (Duovir), & the percentage purity was established to be 99.1% w/v and 98.8% w/v, in conformity with the label claim. This validated novel UV approach could be easily adopted for simultaneous determination of zidovudine and lamivudine in formulations and bulk.

Keywords: Lamivudine and zidovudine, chemometric technique, UV method development and validation.

INTRODUCTION

The combination of lamivudine and zidovudine is used to treat HIV contamination, the virus responsible for AIDS. Nucleoside -reverse transcriptase inhibitor obstruct the HIV virus's reproduction by joining itself to the viral DNA chain [1]. The chemometric method is used for mathematical and statistical analysis, allowing for multidimensional calibration of specific chromatographic, electrochemical, and spectroscopic processes. It is particularly useful for testing pharmaceutical preparations [2]. Design of experiment is a systematic strategy used by engineers & scientists to investigate the rapport among key variable & yield variables [3]. A central composite design can be used in response surface methods to construct a second-order quadratic model for response variables, quantifying the relationship between measured responses and input factors [4]. Parameters followed are preciseness, accuracy, LOD, LOQ, & linearity, as stated via ICH guidelines.

MATERIALS AND METHODS

Drug collection

Lamivudine and zidovudine standard drug was gifted from cipla. Lamivudine 150mg and zidovudine 300mg tablets (Brand: Duovir) was purchased from cipla pharmaceuticals.

Instrument used

Shimadzu BL 220 H Digital balance, UV- Visible spectrophotometer-Perkin Elmer (double beam), melting point apparatus scientific model MP-DS, ultra sonicator PCI Labs, software used is design of expert 13.0 version.

Selection of solvents

Solubility of lamivudine and zidovudine was performed by using solvents like water, ethanol, methanol and DMSO. Based on the good solubility the solvent was selected.

Optimization of method

Preparation of Standard Solution

After weighing and transferring 150 mg of lamivudine and 300 mg of zidovudine standard into two 100 ml volumetric flasks, 50 ml of water was added to each flask, which was then sonicated for 10 minutes and made the final volume. For both Lamivudine and Zidovudine standard, further dilutions were performed until the final concentrations were 10µg/ml.

Preparation of Sample Solution

Tablet powder equivalent to Lamivudine 150mg and Zidovudine 300mg were weighed and transferred into 100ml volumetric flasks, 50ml of water was added to each, sonicated for 10 minutes, and the final volume was made up of water. Finally, the solution was filtered. The filtrate was further diluted to achieve a final concentration of 10µg/ml for Lamivudine and Zidovudine, respectively.

Selection of Absorption Maximum (λ max)

The concentrations of Lamivudine and Zidovudine (10µg/ml) were synthesized and scanned in a UV-Visible spectrometer between 200-400 nm, using distilled water as the blank. The wavelengths for additional chemometric technique investigations were picked from the overlay spectrum.

Optimization of Central Composite Design

The developed UV approach was optimized using the central composite design, which incorporated the use of Design expert. Wavelength 1 (Variable A) and Wavelength 2 (Variable B) were selected as independent variables for statistical analysis; Table 1 displays the low and high levels of independent factors. Response characteristics such as absorbance (A1&A2) from two medications (lamivudine and zidovudine) were employed as robustness parameters.

The statistical computations for variable screening and procedure optimization were done using the Design of Expert program. Thirteen runs were done with two independent variables, which are shown in Table 2.

Validation of Experimental Design

Analysis of variance (ANOVA) was done for each parameter to determine the significance of the effects and interactions between variables. Wavelengths 1 and 2 were chosen as independent variables, and their effects on Absorbance 1 (Lamivudine) and Absorbance 1 (Zidovudine) were assessed. To assess the impact of each response, the response coefficient was calculated for every dependent variable. Significant polynomial response equations for R1 (Absorbance 1) and R2 (Absorbance 2) were generated after each response coefficient was examined and its statistically significant and non-significant response coefficients were eliminated.

Effect of Independent Variables on Response-1 (Absorbance-1)

The effect of independent variables on absorbance-1 (Lamivudine) was expressed by the 3D response counter plot and polynomial equation. Second order quadratic polynomial for response R-1 (Absorbance-1) was varied from 0.3621 to 0.3936. That wavelength 1 (A) negatively affects absorbance-1, while wavelength-2 has no significant impact. The regression coefficients of variables A&B were found to be **0.0045, 0.0000**, respectively. Absorbance-1 decreases with increasing wavelength1 (A), while wavelength-2 have no notable influences on absorbance-1. The positive regression coefficient for AB was evaluated and found to be 0.0000, indicating that response1 (Absorbance 1) had no impact as A and B increased simultaneously.

Effect of Independent Variable on Response-2(Absorbance-2)

The study analyzed the impact of independent variables on absorbance-2 (Zidovudine) using a 3D response, counter plot, and polynomial equation. That wavelength-2 (B) had a positive impact on absorbance-2, while wavelength-1 (A) had no significant influence. The regression coefficients of variables A & B were found to be **0.0000 and 0.0329**, respectively. The experimental model fits well for the method, with adjusted R1 & R2 within an acceptable range (R2 > 0.9). The model's precision value is larger than four, indicating sufficient signal. The coefficient of variation (C.V.) represents repeatability. Three-dimensional plots were used to interpret the interaction, and the maximum desirability function showed an excellent statistical model. ANOVA results showed the actual and predicted response values in Tables 3.

Method validation

Linearity

0.05g of standard Lamivudine and Zidovudine was weighed and dissolved in a distilled water to create a final volume of 1000µg/ml. After that, be diluted by means of distill water to obtain concentration 10-60µg/ml. The absorbance of the solutions was measured at 217nm and 267nm against the blank, and a calibration graph was constructed using concentration vs absorbance.

LOD and LOQ (Limit of Detection & Limit of Quantification)

The LOD & LOQ of the drug lamivudine and zidovudine was calculated using standard deviation as per the ICH guidelines.

Accuracy

A constant amount of pre-analyzed sample was obtained, and the standard medication was administered at 80%, 100%, and 120% concentrations. The standard concentrations for Lamivudine and Zidovudine were established at 10µg/ml. The % recovery was calculated.

Precision

Intraday Precision and Inter-day Precision

0.05g of standard Lamivudine and Zidovudine was weighed and dissolved in water to create a final volume of 1000µg/ml. The solution was diluted to 100µg/ml and 10µg/ml, and the absorbance was measured at 217nm for

Lamivudine and 267nm for Zidovudine at 1 hour intervals and at one day of interval. The % RSD was calculated from the absorbance values which should not be more than 2%.

Ruggedness

i) Preparation (Lamivudine & zidovudine) of six working standard solutions of 10µg/ml by two different analysts were subjected to test absorbance at fixed wavelength in same equipment.

ii) Preparation (Lamivudine & Zidovudine) of three working standard solutions of 10µg/ml by the same analyst and the measurement of absorbance was done at two different systems (Single beam & double beam UV-Visible spectrophotometer).

Estimation of Lamivudine and Zidovudine in Materials Formulation

Preparation of Standard

The process involves transferring 0.05 g of standard lamivudine and 0.05 g of zidovudine in a 50 ml volumetric flask, mixed with distilled water to create a final concentration of 100µg/ml. Then, 1 ml of lamivudine and 2 ml of zidovudine were pipetted into a 10 ml volumetric flask, and the final volume was made up with water to obtain a final concentration of 10 µg/ml of lamivudine and 20 µg/ml of zidovudine.

Preparation of Sample

0.51 g of tablet powder equivalent to 0.1 g of Lamivudine and 0.3 g of Zidovudine were weighed, dissolved in water, and filtered. 1 ml was pipetted out and transferred to a 10 ml volumetric flask, then distilled water was added to obtain the concentration of Lamivudine (10 µg/ml) and Zidovudine (20 µg/ml).

RESULT AND DISCUSSION

Selection of Solvent

The solubility of lamivudine and zidovudine was determined and the purified water was selected as solvent due to it's highly solubility.

Selection of Absorption Maximum (λ max)

Standard solutions of lamivudine 10 µg/ml and zidovudine 10µg/ml in water were prepared, scanned in a UV-visible spectrophotometer and respective UV spectrum was obtained shown in figure 1 and 2, and the wavelength range for optimization (CCD) was selected from the overlay spectrum figure 3.

Optimization by central composite design (CCD)

The central composite design was used to optimize the responses for determining Lamivudine and Zidovudine simultaneously. Thirteen experiments were conducted using Wavelength-1 and Wavelength-2 independent variables to optimize the responses (Absorbance-1 & Absorbance-2) and the results shown in Table 4. Randomized experiments minimized unrestrained variables, and statistical tools verified variables' effects on responses.

Effect of Independent Variable on Response 1 (Absorbance 1)

Independent Variable A and B's Effects on Absorbance 1

When wavelength 1 (A) was increased at a low level (260nm) of wavelength 2, there was no significant change in absorbance-1 lamivudine (less interaction). While increasing wavelength 1 at a high level (270nm) of wavelength 2, absorbance 1 climbs 0.35 to 0.39 and then progressively drops beyond 218.5 nm. This model shows interaction was observed in figure 4 and 5.

Effect of Independent Variable on Response 2 (Absorbance 2)

Independent Variables A and B's Effects on Absorbance 2

There was no discernible change in absorbance 2 (zidovudine) when wavelength 1 was increased at low levels (260 nm) and high levels (270 nm) of wavelength 2. It indicates that factors A and B have little effect on response 2 (absorbance 2). This combination has fewer interactions and a lower risk factor. This model shows interaction was observed in figure 6 and 7.

Validation of the Assay Method

Linearity

The calibration curves for Lamivudine and Zidovudine were obtained in concentration ranges of 5-30μg/ml, showing linear responses and a correlation coefficient of 0.9988 and 0.9999 respectively. Table-7 shows the linearity data of lami & zido. Figure 8 & 9 shows the calibration curvature of lami & zido.

Limit of Detection and Limit of Quantification (LOD & LOQ)

According to ICH guidelines the limit of detection (LOD) of lamivudine and zidovudine was found to be 3.62μg/ml and 1.22μg/ml respectively. Limits of quantification (LOQ) of lami & zidovudine were 10.97μg/ml & 3.69μg/ml correspondingly.

Accuracy

The percentage recovery was determined for the results of accuracy study of the lamivudine and zidovudine. Percent recovery for 80%, 100% & 120% of lamivudine was established to be 99.81, 100.38, and 99.69 respectively. The percentage recovery of 80%, 100%, and 120% of zidovudine was found to be 99.8, 100.60, & 99.84 correspondingly.

Precision

The method's preciseness was established by intra & inter-day analysis. For intra-day lamivudine analysis, the percentage relative standard deviation (%RSD) values were determined to be 1.1645 at 0 hours, 1.2430 at 1 hour, and 0.6720 at 2 hours. The inter-day analysis of lamivudine yielded values of 0.6460 at 0 hours, 1.2085 at 1 hour, and 1.3766 at 2 hours. The intra-day zidovudine analysis, the %RSD values were determined to be 0.5695 at 0 hours, 0.7876 at 1 hour, and 0.4202 at 2 hours. The inter-day analysis of zidovudine yielded values of 0.7180 at 0 hour, 0.8265 at 1 hour and 1.1460 at 2 hours.

Ruggedness

The percentage relative standard deviation of analyst and system variation for lamivudine and zidovudine was shown in the tablet 5 and 6.

Estimation of lamivudine and zidovudine in marketed formulation

The amount present of lamivudine and zidovudine in tablet formulation was found to be 148.76 mg and 296.93 mg respectively. Percent purity of lami and zidovudine in tablet formulation was established to be 99.1% and 98.8% w/v correspondingly.

CONCLUSION

A novel Ultra-Violet assay process was developed for instantaneous evaluation of Lami & Zidovudine in pharmaceutical laboratories. The method used chemometric techniques for optimization of spectroscopic response parameters, resulting in more robust and reliable methods. Central Composite -Design (CCD) was intended for parameter optimization. The method was found to be simple, accurate, and robust, demonstrating the effectiveness of chemometrics in conjunction with UV spectroscopy.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

ACKNOWLEDGMENTS

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REFERENCES

1. Zidovudine and lamivudine for HIV infection: Peter L Anderson, joseph E power. PMCID: PMC2954111 NIHMSID: NIHMS205960 PMID; 20953318
2. El-Gindy A, Hadad GM. Chemometrics in pharmaceutical analysis: an introduction, review, and future perspectives. Journal of AOAC International. 2012 May 1; 95(3):609-23.
3. Manuel Gutierrez J, Leija L, Muñoz R. Bio-Inspired Systems Patents: Electronic Tongues. Recent Patents on Signal Processing (Discontinued). 2012 Sep 1; 2(2):108-14.
4. Singh I, Juneja P, Kaur B, Kumar P. Pharmaceutical applications of chemometric techniques. International Scholarly Research Notices. 2013; 2013.
5. Reguera C, Sánchez MS, Ortiz MC, Sarabia LA. Pareto-optimal front as a tool to study the behaviour of experimental factors in multi-response analytical procedures. Analytica chimica acta. 2008 Aug 29; 624(2):210-22.

Table 1. Experimental Factors and Levels Used in the Central Composite Design

Factor	Name	Level (nm)		
		Low (-)	Medium (0)	High (+)
A	Wavelength 1	213.5	218	223
B	Wavelength 2	257.9	265	272

Table 2. Optimization Method Parameters for Central Composite Experimental Design Validation of Experimental Design

		Factor 1	Factor 2
Std	Run	A : Wavelength 1 (nm)	B: Wavelength 2 (nm)
8	1	218.5	272.07
4	2	222	270
6	3	223.4	265
12	4	218.5	265
7	5	218.5	257.9
13	6	218.5	265
1	7	215	260
5	8	213.5	265
11	9	218.5	265
10	10	218.5	265
3	11	215	270
2	12	222	260
9	13	218.5	265

Table 3. The Predicted and Observed Response Values for the Optimization Condition Method validation

Factor	**Optimized level**	
A: Wavelength 1	217nm	
B:Wavelength 2	267nm	
Response	**Predicted**	**Observed**
R1: Absorbance 1	0.4308	0.4385
R2: Absorbance 2	0.3747	0.3784
Desirability	1	

Table 4. Optimization Method Parameters for Central Composite Experimental Design

		Factor 1	**Factor 2**	**Response 1**	**Response 2**
Std	**Run**	**A:Wavelength 1 (nm)**	**B:Wavelength 2 (nm)**	**R1(Absorbance 1)**	**R2(Absorbance 2)**
8	1	218.5	272.07	0.3936	0.3703
4	2	222	270	0.3365	0.3698
6	3	223.4	265	0.3234	0.3234
12	4	218.5	265	0.3936	0.3621
7	5	218.5	257.9	0.3936	0.2933
13	6	218.5	265	0.3936	0.3234
1	7	215	260	0.3621	0.2925
5	8	213.5	265	0.3127	0.3234
11	9	218.5	265	0.3936	0.3234
10	10	218.5	265	0.3936	0.3234
3	11	215	270	0.3621	0.3698
2	12	222	260	0.3365	0.2925
9	13	218.5	265	0.3936	0.3234

Table 5. Ruggedness Data and System variation of lamivudine

Variation	**Parameter**	**Concentration**	**Absorbance**	**Mean**	**%RSD**
Data- Analyst variation	Analyst-1	10	0.4521	0.4569	0.9213
			0.4598		
			0.4589		
	Analyst-2	10	0.4252	0.4254	1.0121
			0.4299		
			0.4213		
Data-System variation	System-1(Single beam)	10	0.4521	0.4569	0.9213
			0.4598		
			0.4589		
	System-2 (Double beam)	10	0.4224	0.4252	1.2085
			0.4312		
			0.4222		

Table 6. Ruggedness Data and System variation of zidovudine

Variation	**Parameter**	**Concentration**	**Absorbance**	**Mean**	**%RSD**
Data-Analyst variation	Analyst-1	10	0.3563	0.3597	0.4337
			0.3594		
			0.3580		
	Analyst-2	10	0.3857	0.3855	0.8572

			0.3887		
			0.3821		
Data-System variation	System-1(Single beam)	10	0.3563	0.3579	0.4337
			0.3594		
			0.3580		
	System-2 (Double beam)	10	0.3878	0.3843	0.8268
			0.3816		
			0.3835		

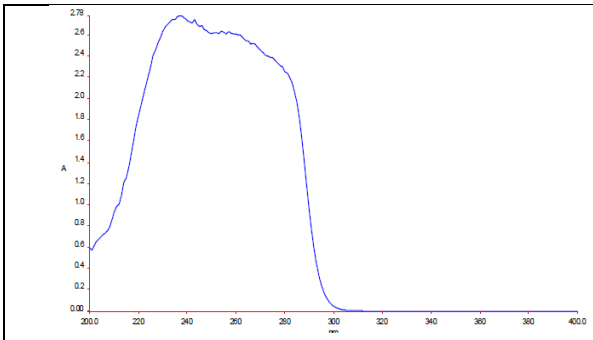

Figure 1. UV-Spectrum of Lamivudine

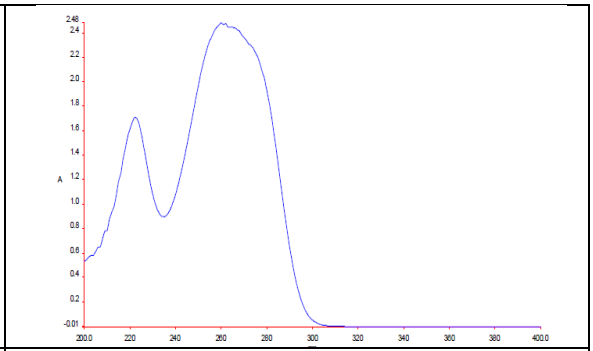

Figure 2. UV-Spectrum of Zidovudine

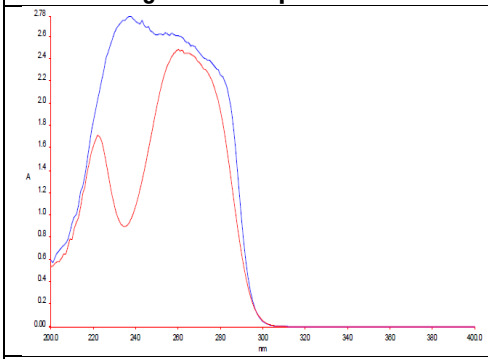

Figure 3. Overlay Spectrum of Lamivudine & Zidovudine

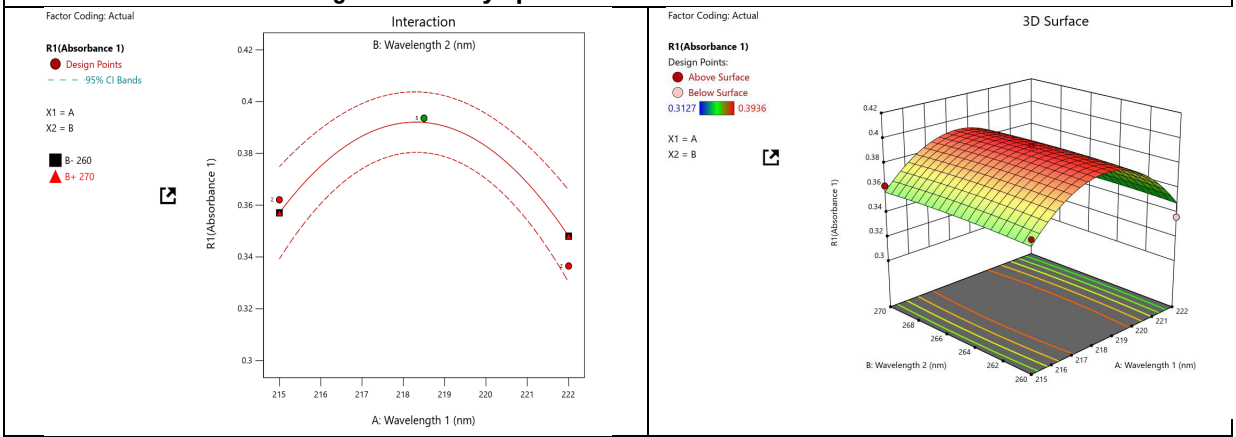

Figure 4 & 5. Interaction &3D Images Showing the Influence of Independent Variables on A&B on Absorbance

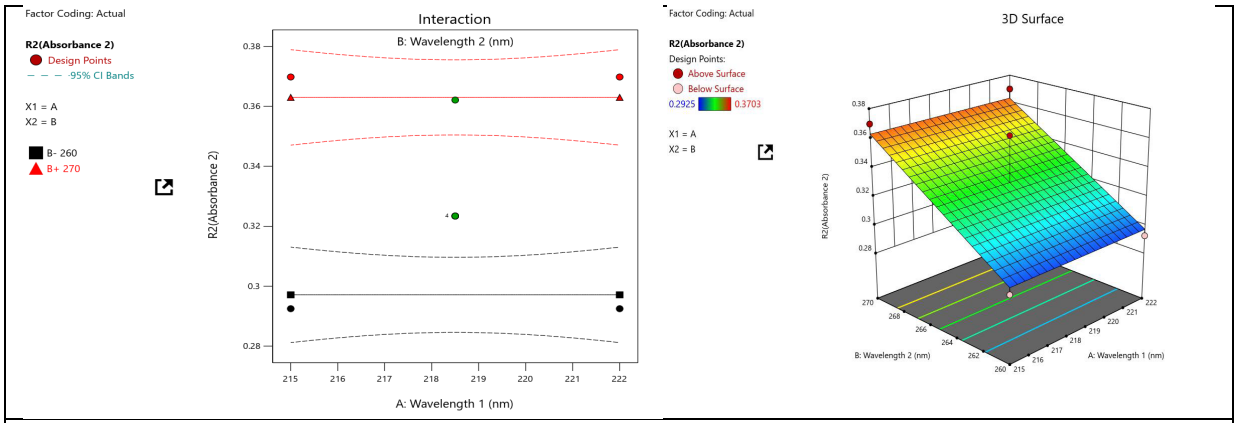

Figure 6 & 7. Interaction & 3D Image Showing the Influence of Independent Variables on A& B on Absorbance 2

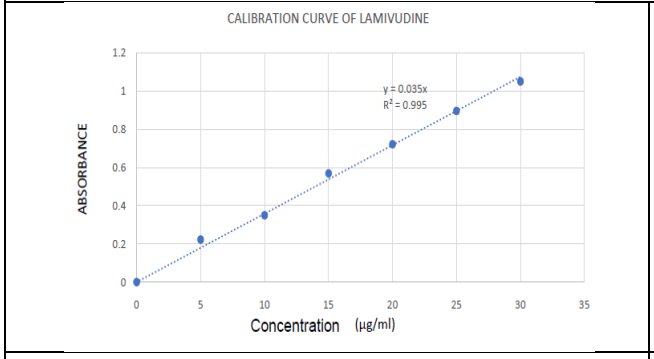

Figure 8. Calibration Curve of Lamivudine

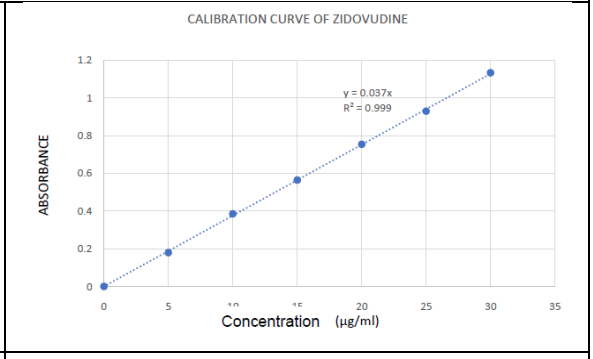

Figure 9. Calibration Curve of Zidovudine